

## Genetic distances among *Oeneis norna*, *O. polixenes*, *O. bore* and *O. melissa* (Satyridae) based on mitochondrial DNA (*ND1*) partial sequences

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**Abstract** Partial sequences (452 bp) of mitochondrial DNA (mtDNA) *ND1* of *Oeneis norna* and *Oeneis melissa* have been determined and compared with reported *ND1* sequences of *Oeneis polixenes*, *O. bore* (*O. taygete*) and *O. uhleri*. It is inferred from the comparison of partial mtDNA sequences that *O. bore*, *O. polixenes*, *O. norna* and *O. melissa* are distinct species to one another and their ancestors were separated at almost the same age. It is mentioned also that at present there is no clear evidence showing that *O. norna* is inhabiting North America including Alaska, which contradicts the descriptions given in a number of illustrated reference books on Japanese butterflies.

**Key words** *Oeneis norna*, *Oeneis polixenes*, *Oeneis bore*, *Oeneis melissa*, Mitochondrial DNA (*ND1*) genes, genetic distance.

### Introduction

The species of genus *Oeneis* are found in the north of the New and Old Worlds. All the species are dull colored, medium in size and principally found in Arctic or alpine habitats as well as in taiga forest. In Japan two *Oeneis* species, *O. norna* and *O. melissa*, distribute, both of which are regarded as high-altitude species inhabiting limited areas above the timberline. The former species inhabits the Hida mountain chain (the Northern Japan Alps) and Mt. Yatsugatake in Honshu Island, while the latter inhabits the Daisetsu mountains and the Hidaka mountain chain in Hokkaido Island. In most of the illustrated reference books published after 1975 in Japan, it is described that *O. norna* ranges both Eurasia and North America (Kawazoé & Wakabayashi, 1976; Fukuda *et al.*, 1984; Matsuka, 1994; Inomata & Matsumoto, 1995; Shirôzu, 2006). It is also written in a foreign illustrated reference book that *O. norna* ranges North America (Higgins & Riley, 1970). However, at present there is no evidence showing that *O. norna* is distributed North America including Alaska, although there are morphological similarities among *norna*, *bore* (*taygete*) and *polixenes*. Although *O. taygete* and *O. bore* were treated recently as the same species (Opler & Wright, 1999; Lukhtanov & Eitschberger, 2001), the relation between *O. norna* and *O. polixenes* is unknown. It is, therefore, of importance to investigate the relationship among the *Oeneis* species. The molecular phylogenetic analyses of the species of genus *Oeneis* have been carried out based on mtDNA (*ND5*) (Usami *et al.*, 2005), but only little information is available for those based on mtDNA (*ND1*). We have investigated the relationship among *norna*, *polixenes*, *bore* (*taygete*) and *melissa* through mtDNA *ND1* (452 bp) gene analyses.

### Materials and methods

Specimen of *O. norna asamana* was obtained in the vicinity of Mt Yakushidake, Japan at altitude near 2,700 m under the permission of Ministry of the Environment, Japan (*cf.*

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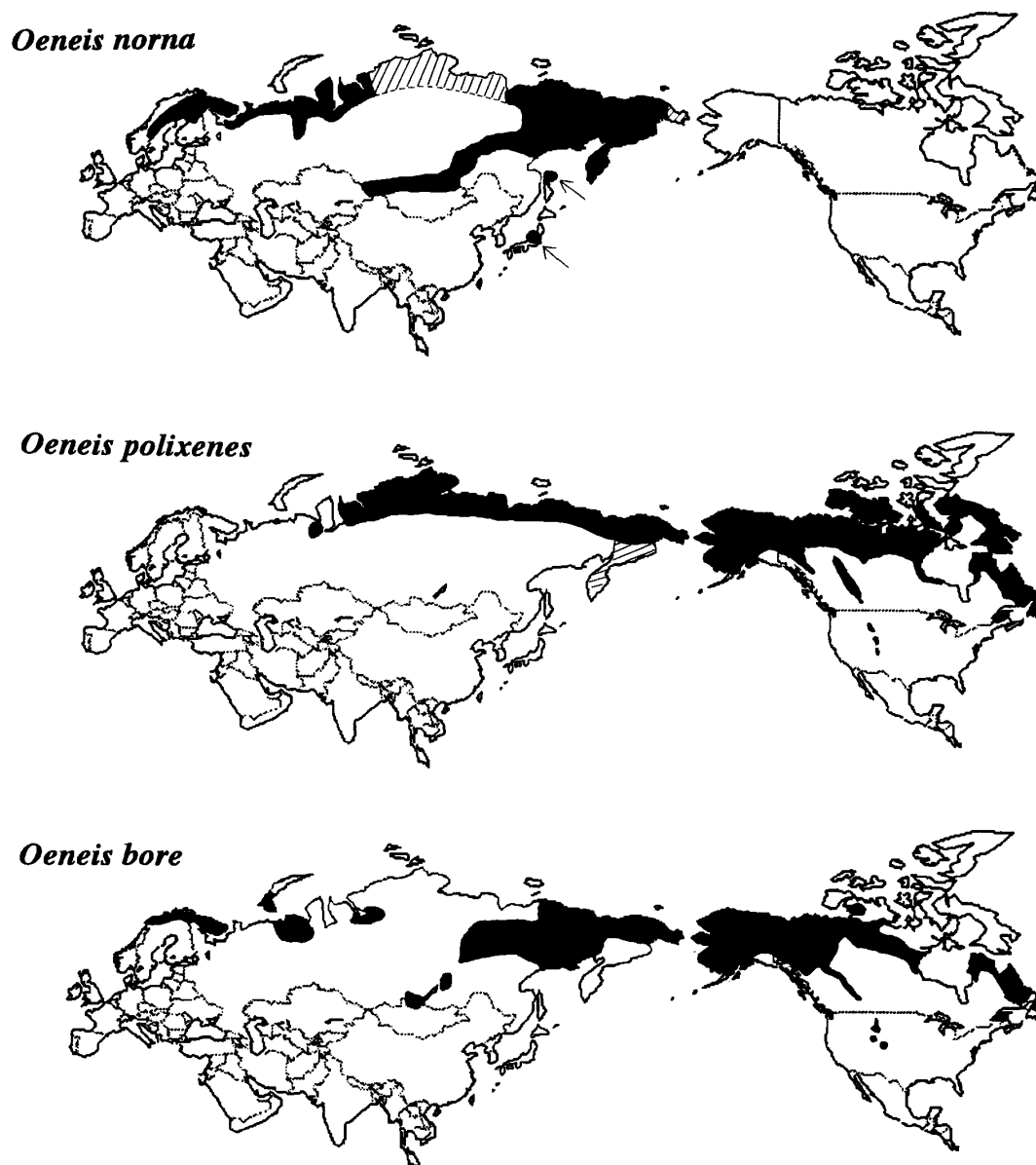


Fig. 1. Outline of the distribution ranges of *Oeneis norna*, *O. polixenes* and *O. bore*. The ranges at Europe were obtained from Higgins and Riley (1970), those in North America were obtained from Opler and Wright (1999), and those in Russia were obtained from Lukhtanov and Eitschberger (2001).

Acknowledgements) in 2001. Specimen of *O. melissa lucilla* has been obtained in the vicinity of the Loveland pass at altitude near 3,800 m in Rocky Mountains, Colorado, while one of the authors (T. Itoh) was staying in Colorado as a visiting Professor of University of Colorado in 2002. These specimens were dropped into ethanol immediately after capturing. MtDNA *ND1* sequences of *O. polixenes*, *O. bore* (*O. taygete*) and *O. uhleri* as well as *Neohipparchia statilinus* used as the reference were obtained from DDBJ data base (Martin *et al.*, 2000).

Total genomic DNA was extracted from male specimens, using protease digestion followed by two phenol-chloroform extractions and ethanol precipitation. Mitochondrial gene region of *NADH dehydrogenase subunit 1* (*ND1*) was applied by PCR using the primers, 5'-CG-TAAAGTCCTAGGTTATATTCAGATTTCG-3' for forward and 5'-ATCAAAGGAGCTC-

Table 1. The mt-DNA (*ND1*) sequences (452 bp) of *Oeneis norna*, *O. polixenes*, *O. bore* and *O. melissa*.

	1	50
<i>O. norna</i>	GGGTTATTACAGCCTTTTCTGATGCTATTAAATTATTTACTAAAGAACA	
<i>O. polixenes</i>	· · T · · · · ·	
<i>O. bore</i>	· · T · · · · ·	
<i>O. melissa</i>	· · T · · · · ·	
	51	100
<i>O. norna</i>	AATTTATTTAAATTATTCAAATTATATTTTATTATTTTCTCCTGTAT	
<i>O. polixenes</i>	· G · · · CC · G · · · · ·	
<i>O. bore</i>	· G · · · · ·	
<i>O. melissa</i>	· G · · · C · · · · ·	
	101	150
<i>O. norna</i>	TAAGATTTATGTTATCTTTAATAATTGGATAGTGATTCCATATTATTTT	
<i>O. polixenes</i>	· · · · · A · · · · ·	
<i>O. bore</i>	· · · · · A · · · · ·	
<i>O. melissa</i>	· · · · · A · · · · · C · · C · · · · ·	
	151	200
<i>O. norna</i>	AATTTAATTGTTTTTAATTTAGGGGTATTATTTTTTTTATGTTGTATAAG	
<i>O. polixenes</i>	· · · · · A · · · · ·	
<i>O. bore</i>	· · · · · C · · · · A · · · · ·	
<i>O. melissa</i>	· · · · ·	
	201	250
<i>O. norna</i>	TTTAGGGGTTTATACTGTTATAATTGCTGGTTGGTCTTCTAATACTAATT	
<i>O. polixenes</i>	· · · · · A · · · · ·	
<i>O. bore</i>	· · · · · A · · · · ·	
<i>O. melissa</i>	· · · · · A · · · · C · · · · ·	
	251	300
<i>O. norna</i>	ATTCTTTATTAGGGGGATTGCGGGCAGTGGCACAGACTATTTCTTATGAA	
<i>O. polixenes</i>	· · · · · A · · · · ·	
<i>O. bore</i>	· · · · · G · · · · A · · · · ·	
<i>O. melissa</i>	· · · · · A · · · · A · · · ·	
	301	350
<i>O. norna</i>	GTTAGTTTAATTTTATTAATATTATCTAGTATTATTTTAATTTTGGATTT	
<i>O. polixenes</i>	· · · · ·	
<i>O. bore</i>	· · · · · G · · · · ·	
<i>O. melissa</i>	· · · · ·	
	351	400
<i>O. norna</i>	TAATTTAATAAAATTTATAGATTATCAGGAATTAATTTGATTTATATTTA	
<i>O. polixenes</i>	· · · · · T · · · · A · · · A · · · ·	
<i>O. bore</i>	· · · · · T · · · · A · · · ·	
<i>O. melissa</i>	· · · · · T · · · · ·	
	401	450
<i>O. norna</i>	TAATATTACCTTTAAGATTTTGTGTTTATGTCTTCTTAATGGCTGAAACT	
<i>O. polixenes</i>	· · · · · A · · · · ·	
<i>O. bore</i>	· · · · · T · · · · A · · · ·	
<i>O. melissa</i>	· · · · · A · · · · A · · · ·	
	451	
<i>O. norna</i>	AA	
<i>O. polixenes</i>	· ·	
<i>O. bore</i>	· ·	
<i>O. melissa</i>	· ·	

GATTAGTTTC-3' for reverse (Aubert *et al.* 1996). Thermal cycle parameter for *ND1* were 2 min at 94°C (1 cycle); 30 s at 94°C, 30 s at 55°C, 1 min at 72°C (30 cycles); 5' min at 72°C (1 cycle); 10 min at 4°C (1 cycle). The PCR product was purified by passing through a gel and fragment sequencing was carried out using a sequence analyzer (ABI Prism 3100).

Table 2. Relative pairwise distance between the *Oeneis* taxa analyzed using Kimura's two-parameter method, along with the standard deviation shown in the parentheses.

	<i>polixenes</i>	<i>bore</i>	<i>melissa</i>	<i>uhleri</i>
<i>norna</i>	0.030 (0.007)	0.029 (0.008)	0.029 (0.008)	0.075 (0.013)
<i>polixenes</i>		0.027 (0.008)	0.023 (0.008)	0.063 (0.012)
<i>bore</i>			0.027 (0.008)	0.065 (0.013)
<i>melissa</i>				0.065 (0.013)

## Results and discussion

In Table 1, we show the sequences of the partial mtDNA *ND1* gene of *O. norna* and *O. melissa*, along with the reported sequences of *O. polixenes* and *O. bore* (*taygete*). Among mtDNA *ND1* sequences of the five *Oeneis* species each consisting of 452 bp, *norna* differs from *polixenes* by 13 bp, differs from *bore* by 13 bp, and differs from *melissa* by 13 bp, while *polixenes* differs from *bore* by 12 bp. The habitat of *polixenes* and *bore* overlaps in part in North America, and these are treated as distinct species (Howe, 1975). In Europe the habitat of *norna* and *bore* also overlaps in part and there *bore* and *norna* are treated as distinct species to each other (Higgins & Riley, 1970). Outlines of the distribution ranges of *norna*, *polixenes* and *bore* are displayed in Fig. 1.

Table 2 shows relative genetic distances between the *Oeneis* taxa analyzed using Kimura's two-parameter method. It is seen in Table 2 that there are no appreciable differences in phylogenetic distance among *norna*, *polixenes*, *bore* and *melissa*, suggesting that ancestors of these four species have separated at almost the same age. Noteworthy is that the distance between *norna* and *melissa*, and that between *norna* and *polixenes* are almost the same, although *norna* belongs to the group different from that of *melissa* or *bore* (Lukhtanov & Eitschberger, 2001). On the other hand, there is a large genetic distance between *O. uhleri* and other four *Oeneis* species, which is consistent with the fact that the morphology of *O. uhleri* is markedly different in size and in color from the four other *Oeneis* species.

In 1988, *Oeneis philipi* inhabiting Alaska very locally was described, which is morphologically almost indistinguishable from *polixenes* (Troubridge *et al.*, 1982; Troubridge & Parshall, 1988). Further, recently *O. taygete* and *O. philipi* were treated as subspecies of *O. bore* and *O. rosovi*, respectively, where *rosovi* is an *Oeneis* species distributing in the far east edge of Eurasia (Opler & Wright, 1999; Lukhtanov & Eitschberger, 2001). Lukhtanov and Eitschberger wrote in a recently published monograph that the male genitalia of *O. philipi* are identical to those of *O. norna*. Therefore, the conspecificity of *O. norna* and *O. philipi* cannot be excluded. According to Layberry *et al.* (1998) *O. philipi* is conspecific with *O. rosovi*. Thus, *O. rosovi*, *O. norna* and *O. philipi* may be treated as the same species in future. However, at present there is no clear evidence showing that *O. norna* is distributed North America including Alaska. This contradicts the descriptions given in illustrated reference books on Japanese butterflies published up to 2006.

## Acknowledgements and notes

The present work is based in part on the survey carried out in 2001 in the vicinity of Mt Yakushi-dake (2,926 m) located at the Northern Japan Alps in Honshu island. The specimens have been obtained under the permission of Ministry of the Environment, Japan with the permission code, Kan-Chubu-Kyo 280 (2001) (T. Itoh). A part of the present results has been published as a DDBJ database (AB182583) in 2004.

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## 摘 要

ミトコンドリア DNA (*ND1*) の部分塩基配列に基づいた *Oeneis norna*, *O. polixenes*, *O. bore* and *O. melissa* の種間の遺伝的距離 (伊藤隆夫・平野和比古・渡辺一雄)

互いに形態的に酷似している4種の *Oeneis* 属種 (*Oeneis norna*, *Oeneis polixenes*, *Oeneis bore*, *Oeneis melissa*) の mtDNA (*ND1*) の452塩基対配列から、これら4種は互いに明瞭な独立種でほぼ同時代に分岐したことが示唆された。また、現在の所、種タカネヒカゲ (*Oeneis norna*) が新大陸に分布している明確な根拠はなく、このことは日本で出版された多数の図鑑の記述と矛盾することを言及した。

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